

CLAIMS

1. A method for determining a target nucleic acid sequence, wherein the target nucleic acid sequence is comprised in a preparation comprising a non-target nucleic acid sequence, the target nucleic acid sequence and the non-target nucleic acid sequence each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the method comprising:

(a) contacting the preparation with an oligonucleotide primer complementary to at least a portion of the first region of common sequence, under conditions to hybridise the primer thereto; and

(b) subjecting the preparation to a sequencing reaction, such that the sequencing reaction proceeds into the second region of dissimilar sequence of the target nucleic acid sequence, thereby determining at least the second region of dissimilar sequence of the target nucleic acid sequence;

and wherein the method further comprises a step of blocking the sequencing reaction between the primer and the non-target nucleic acid sequence, such that the sequencing reaction does not proceed into the second region of dissimilar sequence of the non-target nucleic acid sequence.

2. A method according to claim 1, wherein the target nucleic acid sequence and the non-target nucleic acid sequence each have a second region of common sequence which lies between the first and second regions of dissimilar sequence.

3. A method according to claim 1 or claim 2, wherein the blocking step comprises contacting the preparation with a terminator nucleotide, under conditions to incorporate the terminator nucleotide into the extended or unextended primer hybridised to the non-target nucleic acid sequence but not into the extended or unextended primer hybridised to the target nucleic acid sequence.

4. A method according to claim 3, wherein the conditions are such that the terminator nucleotide is incorporated into substantially all of the extended or unextended primer hybridised to the non-target nucleic acid sequence, before the sequencing reaction reaches the second region of dissimilar sequence.
5. A method according to claim 4, comprising contacting the preparation with the terminator nucleotide after step (a) and before step (b) of claim 1.
6. A method according to claim 4 or claim 5, wherein the terminator nucleotide is complementary to a first nucleotide comprised in the first region of dissimilar sequence of the non-target nucleic acid sequence, but the terminator nucleotide is not complementary to a second nucleotide at a corresponding position in the target nucleic acid sequence.
7. A method according to any of claims 3 to 6, wherein the terminator nucleotide is a dideoxy nucleotide.
8. A method according to claim 7, wherein the terminator nucleotide is capable of covalently cross-linking the primer to the non-target nucleic acid.
9. A method according any preceding claim, wherein the second region of dissimilar sequence comprises a single nucleotide.
10. A method according to any preceding claim, wherein the first region of dissimilar sequence comprises a single nucleotide.
11. A method according to any preceding claim, wherein the sequencing reaction comprises a method of sequencing based on the detection of the release of pyrophosphate.
12. A method according to claim 11, wherein the sequencing reaction comprises pyrosequencing.

13. A method according to any preceding claim, wherein the preparation comprises DNA derived from two or more individuals.

14. A method for determining a plurality of target nucleic acid sequences, wherein the plurality of target nucleic acid sequences is comprised in a preparation further comprising a plurality of corresponding non-target nucleic acid sequences, each target nucleic acid sequence in the preparation corresponds to one or more corresponding non-target nucleic acid sequences in the preparation, each target nucleic acid sequence and each corresponding non-target nucleic acid sequence has a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the first region of common sequence of each target nucleic acid sequence is the same as the first region of common sequence of its corresponding non-target nucleic acid sequences, the first region of dissimilar sequence of each target nucleic acid sequence is different to the first region of dissimilar sequence of its corresponding non-target nucleic acid sequences, the second region of dissimilar sequence of each target nucleic acid sequence is different to the second region of dissimilar sequence of its corresponding non-target nucleic acid sequences, which method comprises:

(a) contacting the preparation with a plurality of oligonucleotide primers, wherein each primer is complementary to at least a portion of the first region of common sequence of a target nucleic acid sequence and its corresponding non-target nucleic acid sequence, under conditions to hybridise the primer thereto; and

(b) subjecting the preparation to a sequencing reaction, such that the sequencing reaction proceeds into the second region of dissimilar sequence of the target nucleic acid sequences, thereby determining at least the second region of dissimilar sequence of each target nucleic acid sequence;

and wherein the method further comprises a step of blocking the sequencing reaction between each primer and each corresponding non-target nucleic acid sequence, such that the sequencing reaction does not proceed into the second region of dissimilar sequence of each corresponding non-target nucleic acid sequence.

15. A method according to any preceding claim, wherein the target nucleic acid sequence and the non-target nucleic acid sequence comprise one or more further regions of dissimilar sequence downstream of the second region of dissimilar sequence.

16. A method for determining the haplotype of a subject from a sample comprising DNA from the subject, comprising a method as defined in any preceding claim, wherein the preparation comprises the sample, the target nucleic acid sequence comprises a locus on a first chromosome of a pair of chromosomes, the non-target nucleic acid sequence comprises the corresponding locus on the second chromosome of the pair, the locus comprising two or more single nucleotide polymorphisms for which the subject is heterozygous, wherein the sequencing reaction is conducted to determine the sequence of the locus on the first chromosome of the pair thereby determining the haplotype of the subject.

17. A method according to claim 16, where the locus comprises a human Class I or Class II HLA gene.

18. Use of pyrosequencing for determining the haplotype of a subject from a sample comprising DNA from the subject, wherein pyrosequencing is used to sequence a target locus on a first chromosome of a pair, the target locus comprising two or more single nucleotide polymorphisms, the corresponding locus on the second chromosome of the pair being blocked from sequencing.

19. Use according to claim 18, wherein the corresponding locus on the second chromosome of the pair is blocked from sequencing by incorporation of a terminator nucleotide into an oligonucleotide primer hybridised to the second chromosome.

20. A kit for determining one or more target nucleic acid sequences, wherein the one or more target nucleic acid sequences are comprised in a preparation comprising one or more non-target nucleic acid sequences, the one or more target nucleic acid sequences and the one or more non-target nucleic acid sequences each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, which kit comprises one or more

oligonucleotide primers complementary to at least a portion of the first region of common sequence and one or more terminator nucleotides.

21. A kit according to claim 20, wherein the terminator nucleotide comprises a dideoxy nucleotide.

22. A kit according to claim 21, wherein the kit includes dideoxy-ATP, dideoxy-CTP, dideoxy-GTP and/or dideoxy-TTP.

23. A kit according to any of claims 20 to 22, further comprising deoxy-ATP, deoxy-CTP, deoxy-GTP, deoxy-TTP, a DNA polymerase, ATP sulfurylase, firefly luciferase and/or a nucleotide-degrading enzyme.